Electropermeabilization versus nsPEF-Stimulation – Pulsed Electric Fields can Stimulate the Growth of Plants and Fungi

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Introduction

Electropermeabilization or Electroporation is a commonly used technique for low temperature cell ingredient extraction like sugar from sugar beets or red pigment from grape skin [1]. When applying pulses of sufficient high electric field and comparable long duration (μs, ms) the cell´s plasma membrane is permeabilized, allowing molecules and ions to pass this natural barrier. In this case the required treatment energy ranges between 10 kJ/kg and 150 kJ/kg and a treatment ordinarily results in cell death. When decreasing treatment energy by reducing field strength and pulse duration to sub-lethal values, recent experiments revealed a stimulating effect on the growth of plants and fungi. For nsPEF-stimulation the pulse duration at a given field strength is chosen to be short enough to prevent considerable plasma membrane permeabilization, which finally preserves cell viability.

Membrane Voltage Measurements

The onset of membrane permeabilization can be detected by membrane voltage measurements using pulsed laser fluorescence microscopy. BY-2 tobacco protoplasts are stained by a fast voltage sensitive dye (ANNINE-6), Fig 1a,b and filled into a microgap which is located on a fluorescence microscope. At the time of interest during the electric field pulse, the cells are illuminated by a 5 ns laser pulse [2]. Hyperpolarization is indicated by an increase of fluorescence intensity, whereas depolarization reduces the intensity, Fig. 1a,b. For a low applied electric field strength, E = 0.2 kV/cm, the azimuthal plot of the membrane voltage V_M shows the typical sinusoidal shape as theoretically expected for an unaffected membrane, Fig. 1c. At a higher field amplitude membrane voltage saturation becomes visible at the hyperpolarized cell hemisphere indicating membrane permeabilization, Fig. 1d. Due to the high plant cell´s resting potential the saturation of V_M at the depolarized cell pole occurs at a higher external electric field. In this case, the resting potential first has to be compensated before the membrane can be recharged to the opposite polarity.

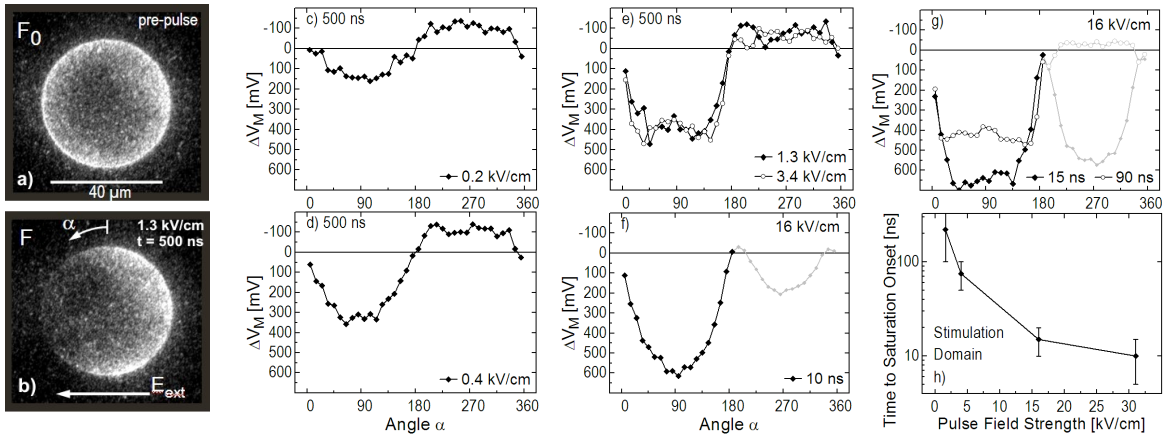

Figure 1: Azimuthal membrane voltage distribution at different time during rectangular electric field pulse application.

At the beginning of a 16 kV/cm electric field pulse, 10 ns after pulse rise, Fig. 1f, the membrane voltage remarkably overshoots the stationary value obtained 500 ns after pulse rise, Fig. 1e. The membrane voltage along the depolarized cell hemisphere is still sinusoidal. Saturation becomes visible 15 ns after pulse rise, Fig. 1g, indicating the onset of membrane permeabilization. 90 ns after pulse rise, Fig. 1g, the saturation value compares well with stationary values at

500 ns indicating a considerable flow of charge carriers through the membrane which finally limits the membrane voltage amplitude to 450 mV. Membrane voltage values for the hyperpolarized hemisphere can not be evaluated from these measurements, since solvatochromatic effects dominate the fluorescence response of the dye for $t < 100$ ns. Fig. 1h summarizes the onset time of membrane voltage saturation associated with enhanced membrane permeabilization for different amplitudes of the applied electric field square pulse. Despite differences in cell diameter, these values compare well with findings from stimulation experiments.

nsPEF-Stimulation – Results and Discussion

Experiments were performed on 3 days old seedlings of *Arabidopsis thaliana* [3]. The seedlings were exposed to electric pulses with a duration of 10 ns, 25 ns and 100 ns. The grown leaf area was evaluated 5 days after nsPEF treatment, Fig. 2a,b. In all cases, where membrane voltage measurements revealed voltage saturation, i.e. enhanced membrane permeability, seedling growth was reduced, compared to the control. When applying 10 ns pulses the grown leaf area was larger or equal compared to the control up to a field strength of 50 kV/cm, whereas for 100 ns pulses a comparable stimulation only could be obtained for 5 kV/cm pulses, Fig. 2c. Seedlings exposed to higher amplitude 100 ns pulses exhibited reduced growth or completely died. Obviously, the stimulating effect is masked by the growth inhibiting impact of membrane permeabilization.

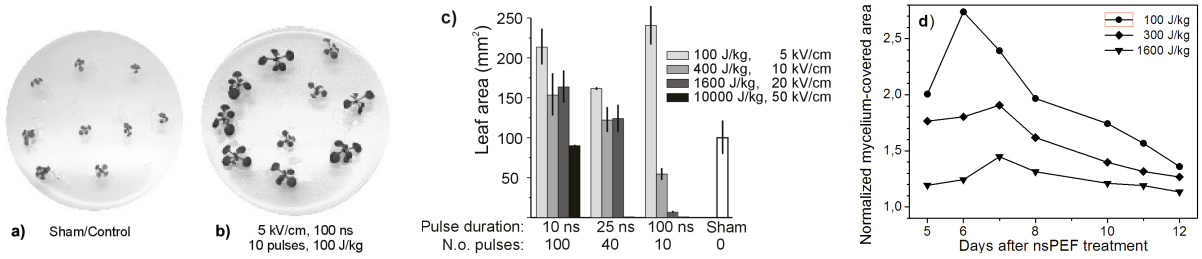

Figure 2: nsPEF-stimulation of *Arabidopsis thaliana*, a-c, and mycelium growth of *Hypsizygus ulmaris,* d.

These results were confirmed by mycelium growth of *Hypsizygus ulmaris*. After treating germinated spores by 100 ns, 5kV/cm pulses, a maximum of growth stimulation of a factor of 2.8 could be obtained, Fig. 2d. These experiments further show the temporal dynamics of the stimulation, exhibiting a maximum at 6-7 days after nsPEF-stimulation. As already shown by Tsukamoto [4] the fruiting body yield from nsPEF-treated mycelium in our case was more than twice compared to the control.

For clarification of the biological and biophysical basics of nsPEF-stimulation the nuclear position of BY-2 cells one day after treating them with 25ns, 10kV/cm pulses was determined. Prior to mitosis, the nucleus of BY-2 cells is moved from the cell wall to the centre of the cell. The rate of pulsed cells exhibiting its nucleus in the centre of the cell was 30 %, whereas for untreated BY-2 cells only a rate of 16 % could be obtained. Furthermore, the mitotic index of nsPEF-treated BY-2 cells, i.e. the relative number of cells undergoing mitosis, was almost twice compared to untreated cells. These results suggest that nsPEF-stimulation accelerates the onset of mitosis.

The cytoskeleton is mainly involved in cell mitosis. A single 10 ns, 30kV/cm pulse results in complete dissolution of actin cytoskeleton filaments. As a consequence of nsPEF treatment actin filaments retract from the membrane region and concentrate around the nucleus [5]. Our current working hypothesis is that actin cytoskeleton dissolution and subsequent reorganization triggers mitosis, which might result in increased organism growth. A comprehensive explanation for nsPEF-stimulation cannot be given at the current state of work. The phenomenon of growth stimulation by nanosecond pulsed electric fields is evident, but far away from being understood. Nevertheless, the low treatment energy required for stimulation, 0.1 kJ/kg to 0.5 kJ/kg, is promising for biotechnological applications.

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